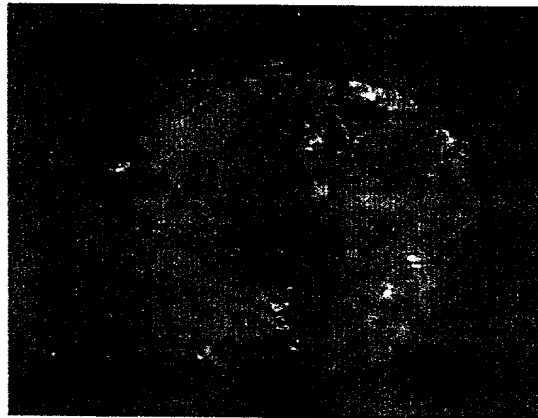E16

FIG. 1A

P60

FIG. 1B

← 1018 bp

← 507 bp

FORWARD PRIMER [GCGGGGCGGTGCGTGACTAC]
REVERSE PRIMER [GGGTGGTGAGGGGTTGAGGTTTGTG]
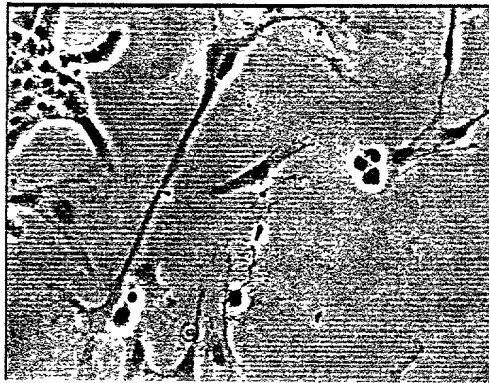
FIG. 2

NESTIN POSITIVE CELLS PROLIFERATE AROUND ISLETS IN VITRO
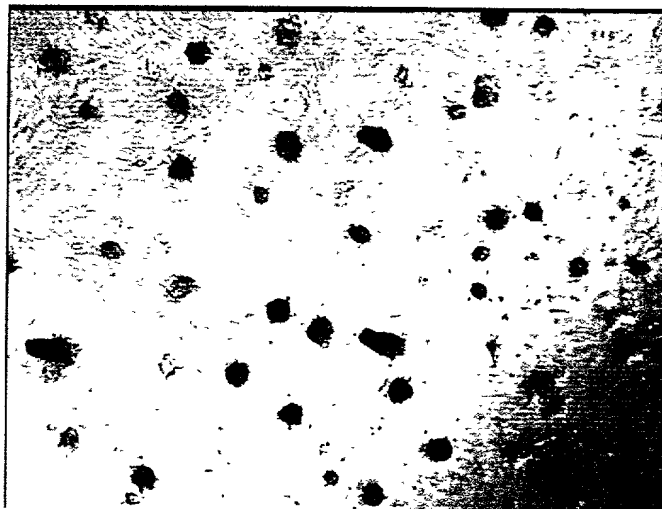

FIG. 3

100x

FIG. 4A

200x

FIG. 4B

FIG. 5

APRT

	LOW GLUCOSE	HIGH GLUCOSE
CYCLE	26 27	26 27
RATIO HIGH/LOW GLUCOSE	1.74	1.69

NESTIN

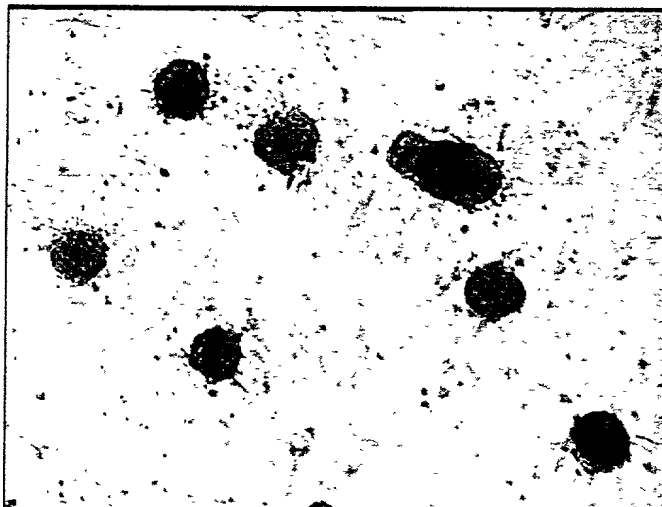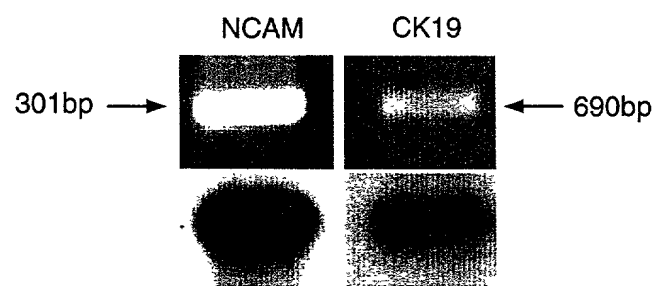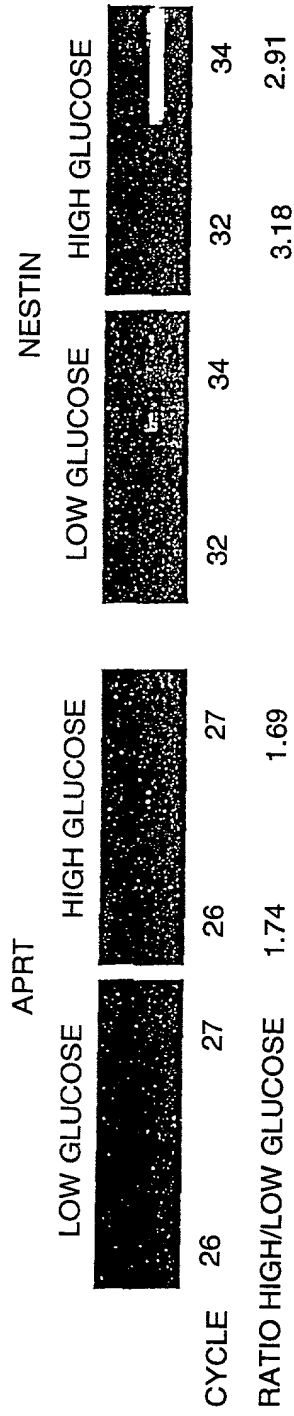|	LOW GLUCOSE	HIGH GLUCOSE
CYCLE	32 34	32 34
RATIO HIGH/LOW GLUCOSE	3.18	2.91

FIG. 6

Nestin Amino Acid Sequence:

"MEGCMGEESFQMWELNRRLEAYLGRVKALEEQNELLSAGLGGLR
RQSADTSWRAHADDELAALRALVDQRWREKHAAEVARDNLAEELEGVAGRCEQLRL
ARERTTEEVARNRRAVEAEKCARAWLSSQGAELERELEALRVAHEEERVGLNAQAAC
APRLPAPPRPPAPAPEVEELARRLGEAWRGAVRGYQERVAHMETSLDQTRERLARAVQ
GAR
EVRLELQQLQAERGGLLERRAALEQRLEGRWQERLRATEKFQLAVEALEQEKQGLQSQ
IAQVLEGRQQLAHLKMSLSLEVATYRTLLEAENSRLQTPGGGSKTSLSFQDPKLELQF
PRTPEGRRLGSLLPVLSPTSLPSPLPATLETPVPAFLKNQEFLQARTPTLASTPIPPT
PQAPSPAVDAEIRAQDAPLSLLQTQGGRKQAPEPLRAEARVAIPASVLPGPEEPGGQR
QEASTGQSPEDHASLAPPLSPDHSSLEAKDGESGGSRVFSICRGEGEGQIWGLVEKET
AIEGKVVSSLQQEIWEEDLNRKEIQDSQVPLEKETLKSLGEEIQESLKTLENQSHET
LERENQECPRSLEEDLETLKSLEKENKRAIKGCGGSETSRKRGCRQLKPTGKEDTQTL
QSLQKENQELMKSLEGNLETFLFPGTENQELVSSLQENLESLTALEKENQEPLRSPEV
GDEEALRPLTKENQEPLRSLEDENKEAFRSLEKENQEPLKTLEEEDQSIVRPLETENH
KSLRSLEEQDQETLRTLEKETQQRRRSLGEQDQMTLRPPEKVDLEPLKSLDQEIARPL
ENENQEFLKSLKEESVEAVKSLETEILESLKSAGQENLETLKSPETQAPLWTPEEINK
SGGNESSRKGNSRTTGVCGSEPRDIQTPGRGESGIIEISGSMEPGEFEISRGVDKESQ
RNLEEEENLGKGEYQESLRSLEEEGQELPQSADVQRWEDTVEKDQELAQESPPGMAGV
ENKDEAELNLREQDGFTGKEEVVEQGELNATEEVWFPGEGHPENPEPKEQRGLVEGAS
VKGGAEGLQDPEGQSQQVGTPGLQAPQGLPEAIEPLVEDDVAPGGDQASPEVMLGSEP
AMGESAAGAEPGLGQGVGGLGDPGHLTREEVMEPPLEEESLEAKRVQGLEGPRKDLEE
AGGLGTEFSELPGKSRDPWEPPREGREESEAEAPRGAEEAFPAETLGHTGSDAPSPWP
LGSEEAEEDVPPVLVSPSPTYTPILEDAPGLQPQAEGSQEASWGVQGRAEAGKVESEQ
EELGSGEIPEGLQEEGEESREESEEDELGETLPDSTPLGFYLRSPTSPRWTPLESRGH
PLKETGKEGWDPAVLASEGLEEPSEKEEGEEGEEECGRDSDLSEEFEDLGTEAPFLPG
VPGEVAEPLGQVPQLLLDPAAWDRDGESDGFADEEESGEEGEEDQEEGREPGAGRWGP
GSSVGSLQALSSSQRGEFLESDSVSVSVPWDDSLRGAVAGAPKTALETESQDSAEPSG
SEEESDPVSLEREDKVPGPLEIPSGMEDAGPGADIIGVNGQGPNLEGKSQHVNGGVMN
GLEQSEESGARNALVSEGDRGSPFQEEEGSALKRSSAGAPVHLGQGQFLKFTQREGDR
ESWSSGED"

Nestin Nucleotide Sequence:
BASE COUNT 1238 a 1176 c 1676 g 764 t ORIGIN 1
atggagggct gcatgggga ggagtcgttt cagatgtggg agctcaatcg gcgcctggag 61
gcctacctgg gccgggtcaa ggcgctggag gagcagaatg agctgctcag cgccggactc 121
gggggctcc ggcgacaatc cgcggacacc tcctggcggg cgcatgccga cgacgagctg 181
gcggccctgc gtgcgctcgt tgaccaacgc tggcgggaga agcacgcggc cgaggtggcg 241
cgcgacaacc tggctgaaga gctggagggc gtggcaggcc gatgcgagca gctgcggctg 301
gcccgggagc ggacgacgga ggaggtagcc cgcaaccggc gcgccgtcga ggcagagaaa
361 tgcgcccggg cctggctgag tagccagggg gcagagctgg agcgcgagct agaggctcta
421 cgcgtggcgc acgaggagga gcgcgtcggt ctgaacgcgc aggctgcctg tgccccccgc
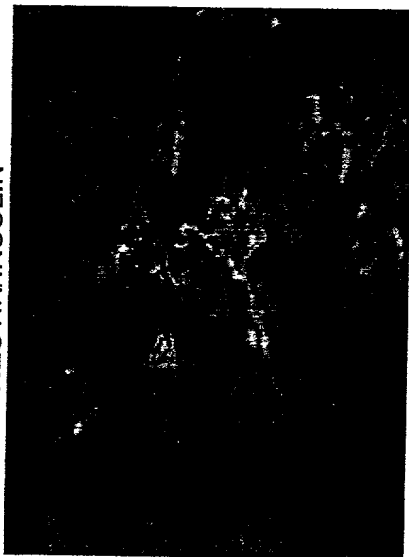
FIG. 7A

481 ctgcccgcgc cgcccggcc tcccgcgccg gccccggagg tagaggagct ggcaaggcga
541 ctgggcgagg cgtggcgcgg ggcagtgcgc ggctaccagg agcgcgtggc acacatggag
601 acgtcgctgg accagacccg cgagcgcctg gcccgggcgg tgcagggtgc ccgcgaggtc
661 cgcctggagc tgcagcagct ccaggctgag cgcggaggcc tcctggagcg cagggcagcg
721 ttggaacaga ggttggaggg ccgctggcag gagcggctgc gggctactga aaagttccag
781 ctggctgtgg aggccctgga gcaggagaaa cagggcctac agagccagat cgctcaggtc
841 ctggaaggtc ggcagcagct ggcgcacctc aagatgtccc tcagcctgga ggtggccacg
901 tacaggaccc tcctggaggc tgagaactcc cggctgcaaa cacctggcgg tggctccaag
961 acttccctca gctttcagga ccccaagctg gagctgcaat ccctaggac cccagagggc
1021 cggcgtcttg gatctttgct cccagtcctg agcccaactt ccctcccctc acccttgcct
1081 gctacccttg agacacctgt gccagccttt cttaagaacc aagaattcct ccaggcccgt
1141 acccctacct tggccagcac ccccatcccc ccacacctc aggcaccctc tcctgctgta
1201 gatgcagaga tcagagccca ggatgctcct ctctctctgc tccagacaca gggtgggagg
1261 aaacaggctc cagagcccct gcgggctgaa gccagggtgg ccattcctgc cagcgtcctg
1321 cctggaccag aggagcctgg gggccagcgg caagaggcca gtacaggcca
gtccccagag 1381 gaccatgcct ccttggcacc acccctcagc cctgaccact ccagtttaga
ggctaaggat 1441 ggagaatccg gtgggtctag agtgttcagc atatgccgag gggaaggtga
agggcaaatc 1501 tgggggttgg tagagaaaga aacagccata gagggcaaag tggtaagcag
cttgcagcag 1561 gaaatatggg aagaagagga tctaaacagg aaggaaatcc aggactccca
ggttcctttg 1621 gaaaaagaaa ccctgaagtc tctgggagag gagattcaag agtcactgaa
gactctggaa 1681 aaccagagcc atgagacact agaaagggag aatcaagaat gtccgaggtc
tttagaagaa 1741 gacttagaaa cactaaaaag tctagaaaag gaaaataaaa gagctattaa
aggatgtgga 1801 ggtagtgaga cctctagaaa aagaggctgt aggcaactta gcctacagg
aaaagaggac 1861 acacagacat tgcaatccct gcaaaaggag aatcaagaac taatgaaatc
tcttgaaggt 1921 aatctagaga cattttttatt tccaggaacg gaaaatcaag aattagtaag
ttctctgcaa 1981 gagaacttag agtcattgac agctctggaa aaggagaatc aagagccact
gagatctcca 2041 gaagtagggg atgaggaggc actgagacct ctgacaaagg agaatcagga
acccctgagg 2101 tctcttgaag atgagaacaa agaggccttt agatctctag aaaaagagaa
ccaggagcca 2161 ctgaagactc tagaagaaga ggaccagagt attgtgagac ctctagaaac
agagaatcac 2221 aaatcactga ggtctttaga agaacaggac caagagacat tgagaactct
tgaaaaagag 2281 actcaacagc gacggaggtc tctaggggaa caggatcaga tgacattaag
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tcttgaaaat 2401 gagaatcaag agttcttaaa gtcactcaaa gaagagagcg tagaggcagt
aaaatcttta 2461 gaaacagaga tcctagaatc actgaagtct gcgggacaag agaacctgga
aacactgaaa 2521 tctccagaaa ctcaagcacc actgtggact ccagaagaaa taaataaatc
aggggggcaat 2581 gaatcctcta gaaaaggaaa ttcaagaacc actggagtct gtggaagtga
accaagagac 2641 attcagactc ctggaagagg agaatcagga atcattgaga tctctgggag
catggaacct 2701 ggagaatttg agatctccag aggagtagac aaggaaagtc aaaggaatct
ggaagaggaa 2761 gagaacctgg aaagggaga gtaccaagag tcactgaggt ctctggagga
ggagggacag 2821 gagctgccgc agtctgcaga tgtgcagagg tgggaagata cggtggagaa
ggaccaagaa 2881 ctggctcagg aaagccctcc tgggatggct ggagtggaaa ataaggatga
ggcagagctg 2941 aatctaaggg agcaggatgg cttcactggg aaggaggagg tggtagagca
gggagagctg 3001 aatgccacag aggaggtctg gttcccaggc gaggggcacc

FIG. 7B

cagagaaccc tgagcccaaa 3061 gagcagagag gcctggttga gggagccagt
gtgaagggag gggctgaggg cctccaggac 3121 cctgaagggc aatcacaaca
ggtggggacc ccaggcctcc aggctcccca ggggctgcca 3181 gaggcgatag agcccctggt
ggaagatgat gtggccccag ggggtgacca agcctcccca 3241 gaggtcatgt tggggtcaga
gcctgccatg ggtgagtctg ctgcgggagc tgagccaggc 3301 ctggggcagg gggtggggagg
gctggggggac ccaggccatc tgaccaggga agaggtgatg 3361 gaaccacccc
tggaagagga gagtttggag gcaaagaggg ttcagggctt ggaagggcct 3421 agaaaggacc
tagaggaggc aggtggtctg gggacagagt tctccgagct gcctgggaag 3481 agcagagacc
cttgggagcc tcccagggag ggtagggagg agtcagaggc tgaggccccc 3541
aggggagcag aggaggcgtt ccctgctgag accctgggcc acactggaag tgatgcccct 3601
tcaccttggc ctctggggtc agaggaagct gaggaggatg taccaccagt gctggtctcc 3661
cccagcccaa cgtacacccc gatcctggaa gatgccctg ggctccagcc tcaggctgaa 3721
gggagtcagg aggctagctg ggggggtgcag gggagggctg aagctgggaa agtagagagc 3781
gagcaggagg agttgggttc tggggagatc cccgagggcc tccaggagga aggggaggag 3841
agcagagaag agagcgagga ggatgagctc ggggagaccc ttccagactc cactccctg 3901
ggcttctacc tcaggtcccc cacctccccc aggtggaccc cactggagag cagaggccac 3961
cccctcaagg agactggaaa ggagggctgg gatcctgctg tcctggcttc cgagggcctt 4021
gaggaaccct cagaaaagga ggagggggag gagggagaag aggagtgtgg·ccgtgactct
4081 gacctgtcag aagaatttga ggacctgggg actgaggcac cttttcttcc tggggtccct
4141 ggggaggtgg cagaacctct gggccaggtg ccccagctgc tactggatcc tgcagcctgg
4201 gatcgagatg gggagtctga tgggtttgca gatgaggaag aaagtgggga ggagggagag
4261 gaggatcagg aggaggggag ggagccaggg gctgggcggt ggggggccagg gtcttctgtt
4321 ggcagcctcc aggccctgag tagctcccag agagggggaat tcctggagtc tgattctgta
4381 agtgtcagcg tcccctggga tgacagcttg aggggtgcag tggctggtgc ccccaagact
4441 gccctggaaa cggagtccca ggacagtgct gagccttctg gctcagagga agagtctgac
4501 cctgtttcct tggagagggga ggacaaagtc cctggccctc tagagatccc cagtgggatg
4561 gaggatgcag gcccaggggc agacatcatt ggtgttaatg gccagggtcc caacttggag
4621 gggaagtcac agcatgtaaa tgggggagta atgaacgggc tggagcagtc tgaggaaagt
4681 ggggcaagga atgcgctagt ctctgaggga gaccgaggga gcccctttca ggaggaggag
4741 gggagtgctc tgaagaggtc ttcggcaggg gctcctgttc acctgggcca gggtcagttc
4801 ctgaagttca ctcagaggga aggagataga gagtcctggt cctcagggga ggac //
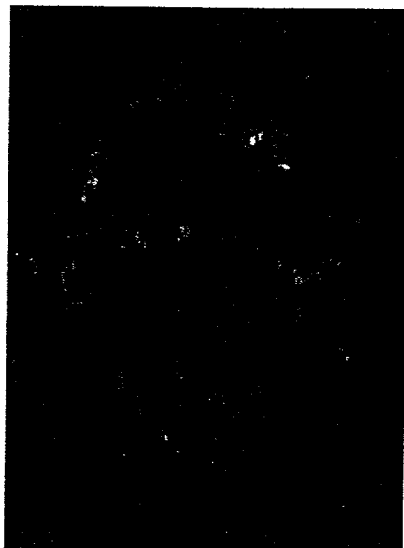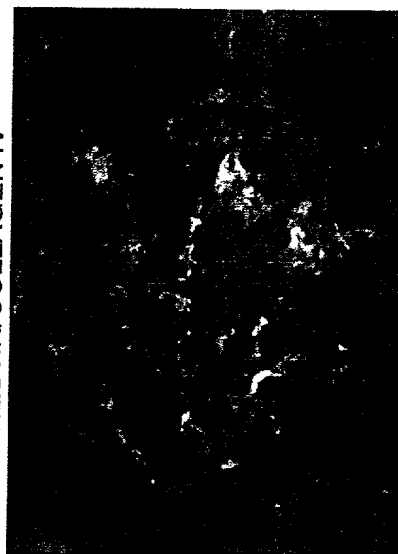
FIG. 7C

NESTIN/INSULIN

E16
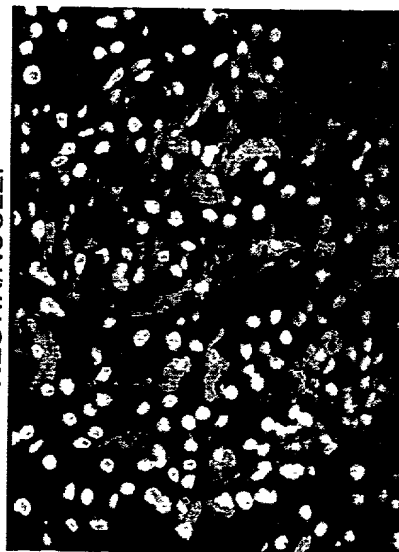
FIG. 8A

NESTIN/INSULIN

P60

FIG. 8B

NESTIN/COLLAGEN IV

P60

FIG. 8C

NESTIN/NUCLEI

P60

FIG. 8D

NESTIN

RAT　　HUMAN

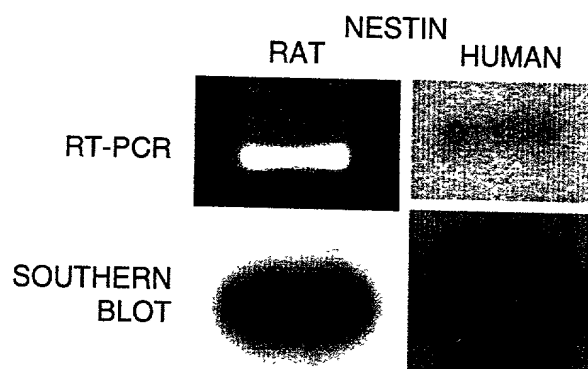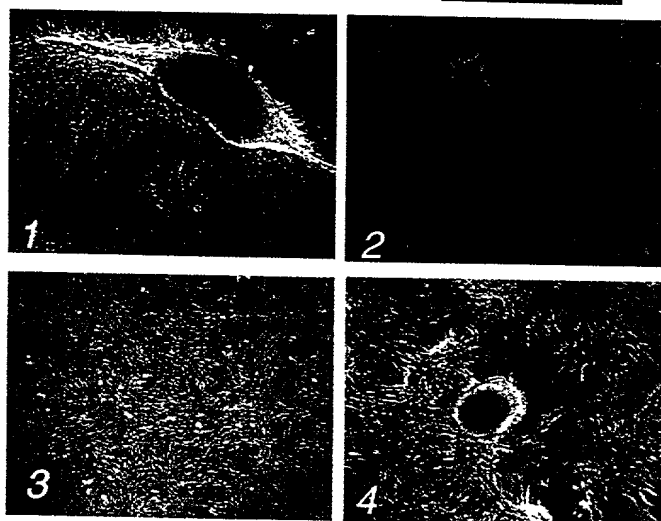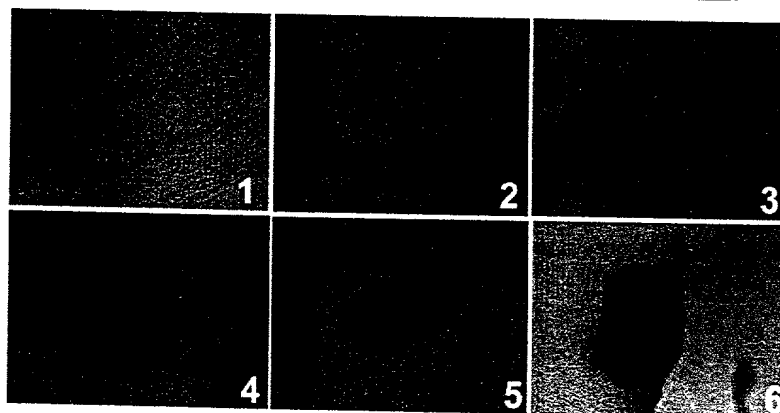RT-PCR

SOUTHERN
BLOT

FIG. 8E

FIG. 9A

FIG. 9B

CK19

RAT　　HUMAN　　NCAM
　　　　　　　　　RAT

RT-PCR

SOUTHERN
BLOT

FIG. 9C

FIG. 10A

IDX

RAT

RT-PCR

SOUTHERN
BLOCK

FIG. 10B

NIPC
(HUMAN)   INS1

WESTERN
BLOCK-IDX

PRE-
IMMUNE

FIG. 10C

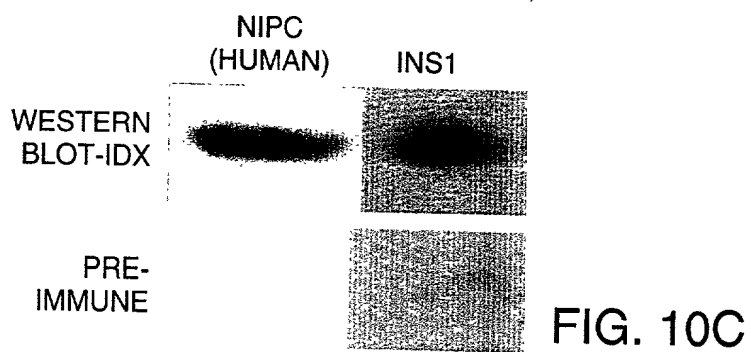PROGLUCAGON

ILCs   ISLETS
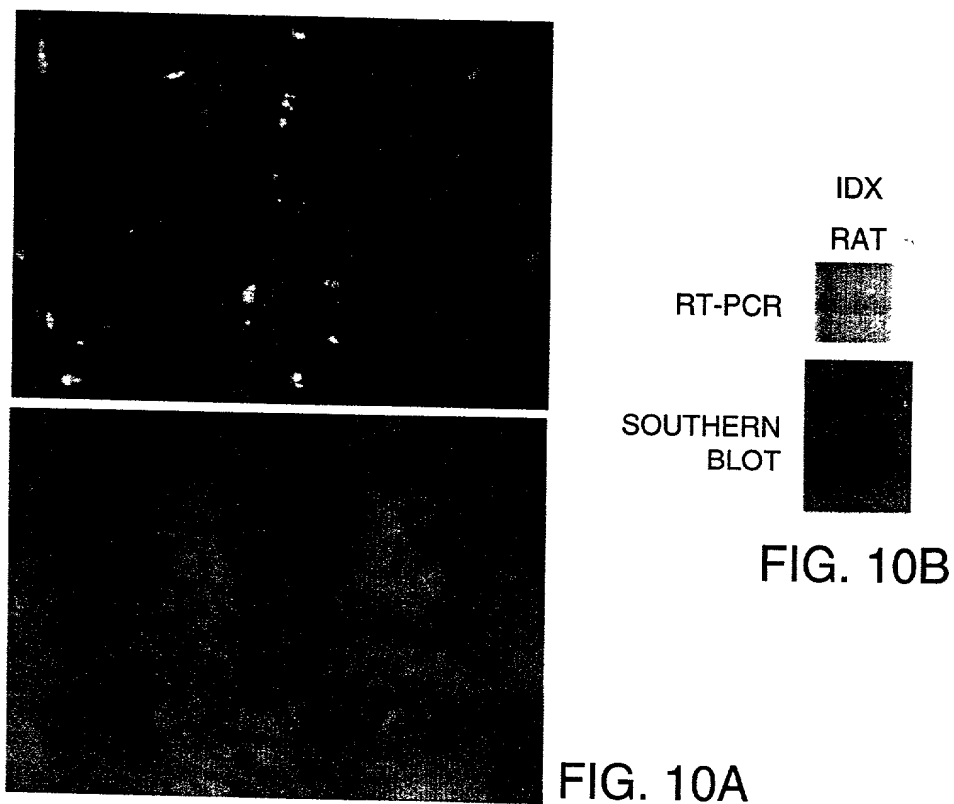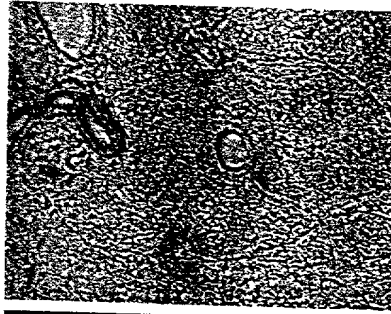
RT-PCR

SOUTHERN
BLOCK

FIG. 10D

CK19 / NESTIN
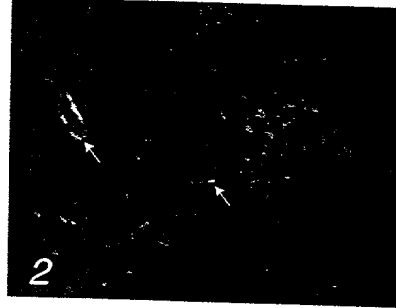
FIG. 11A

CK19 / NESTIN

FIG. 11B

NESTIN

NESTIN/NUCLEI
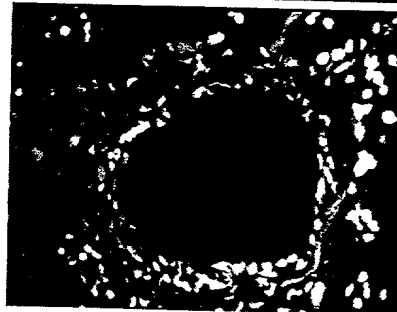
FIG. 11C

MODEL 1

ENDOCRINE CELL

MORPHOGENS

SPECIALIZED REGIONS OF
EPITHELIAL CELLS

DUCT
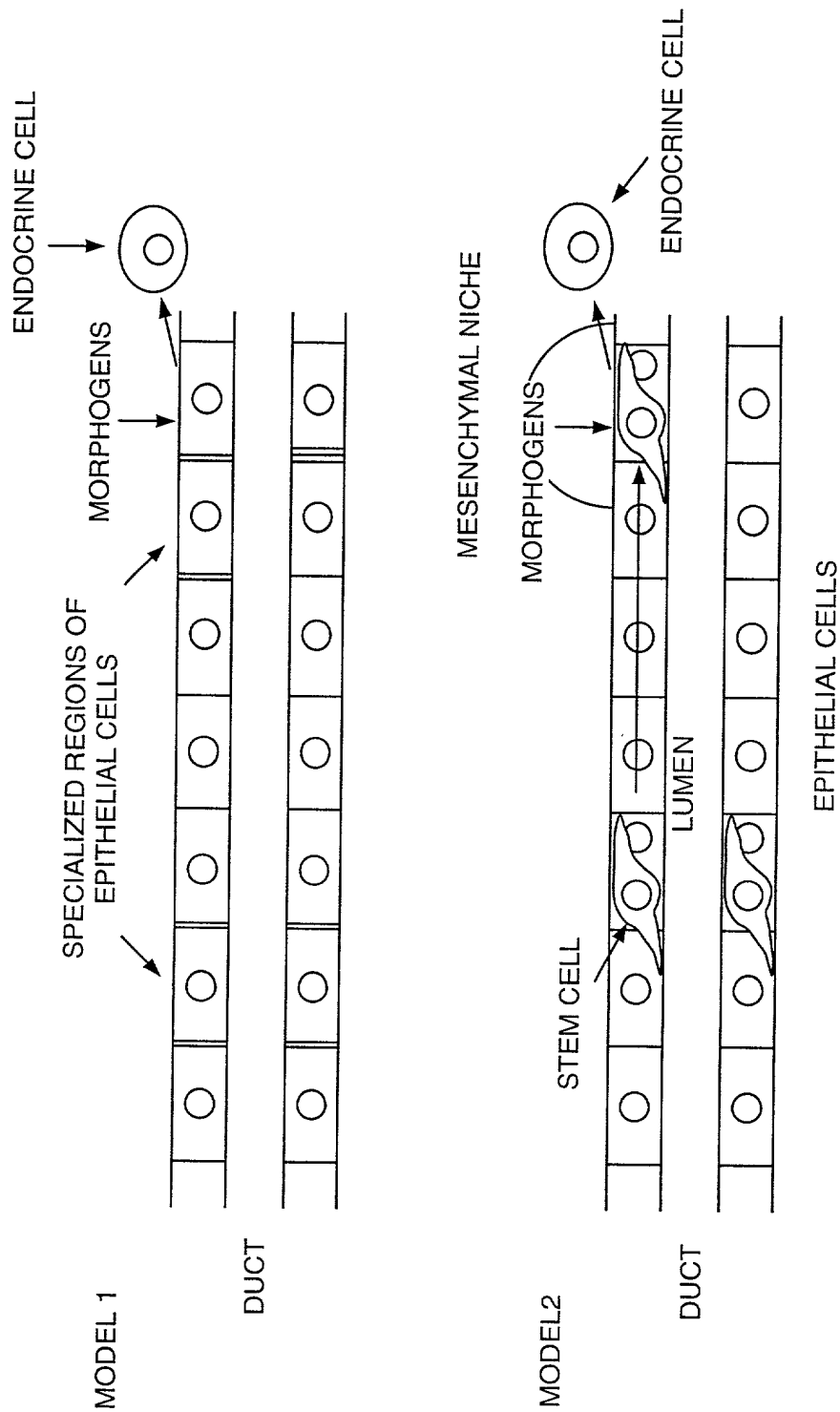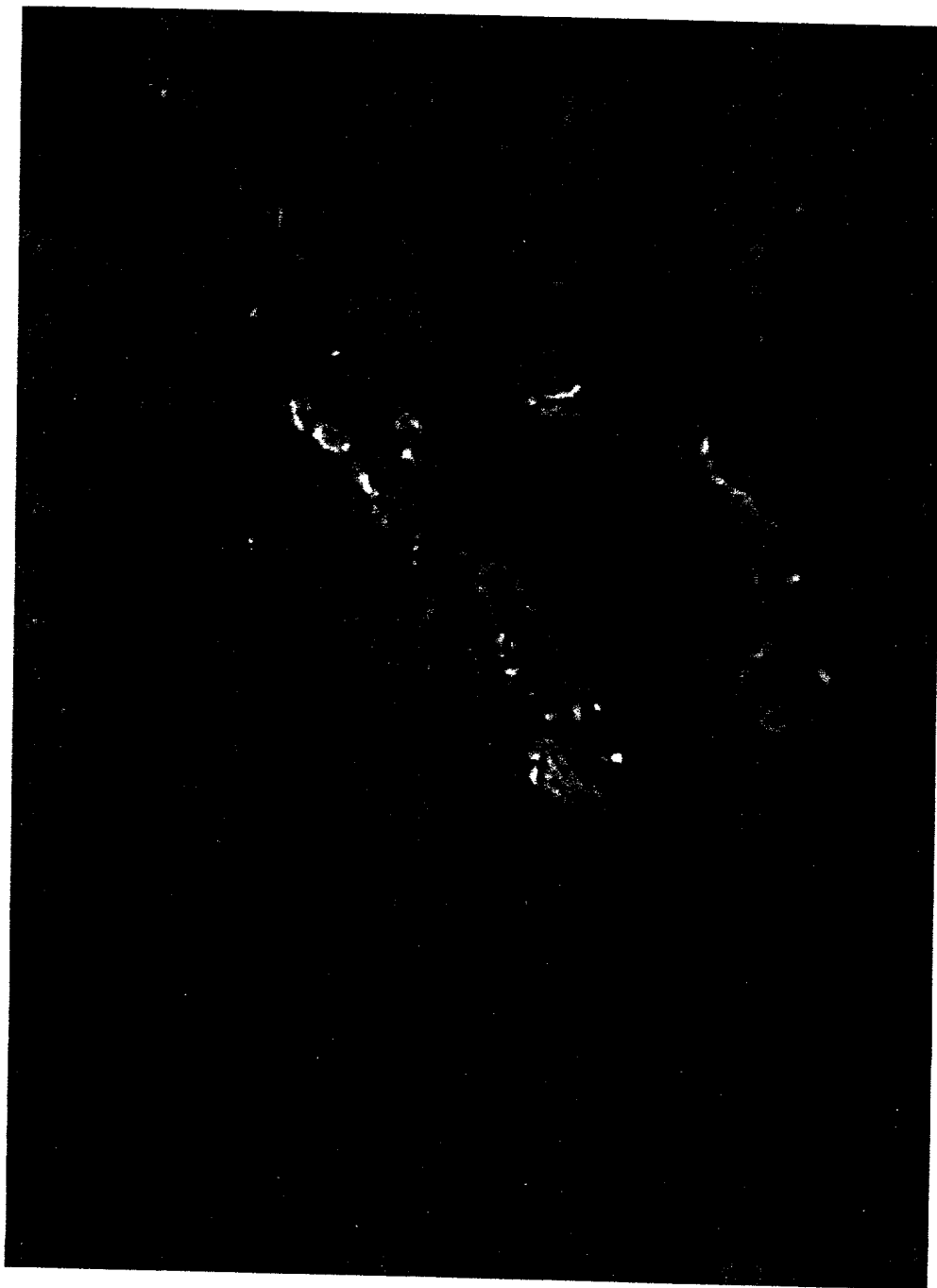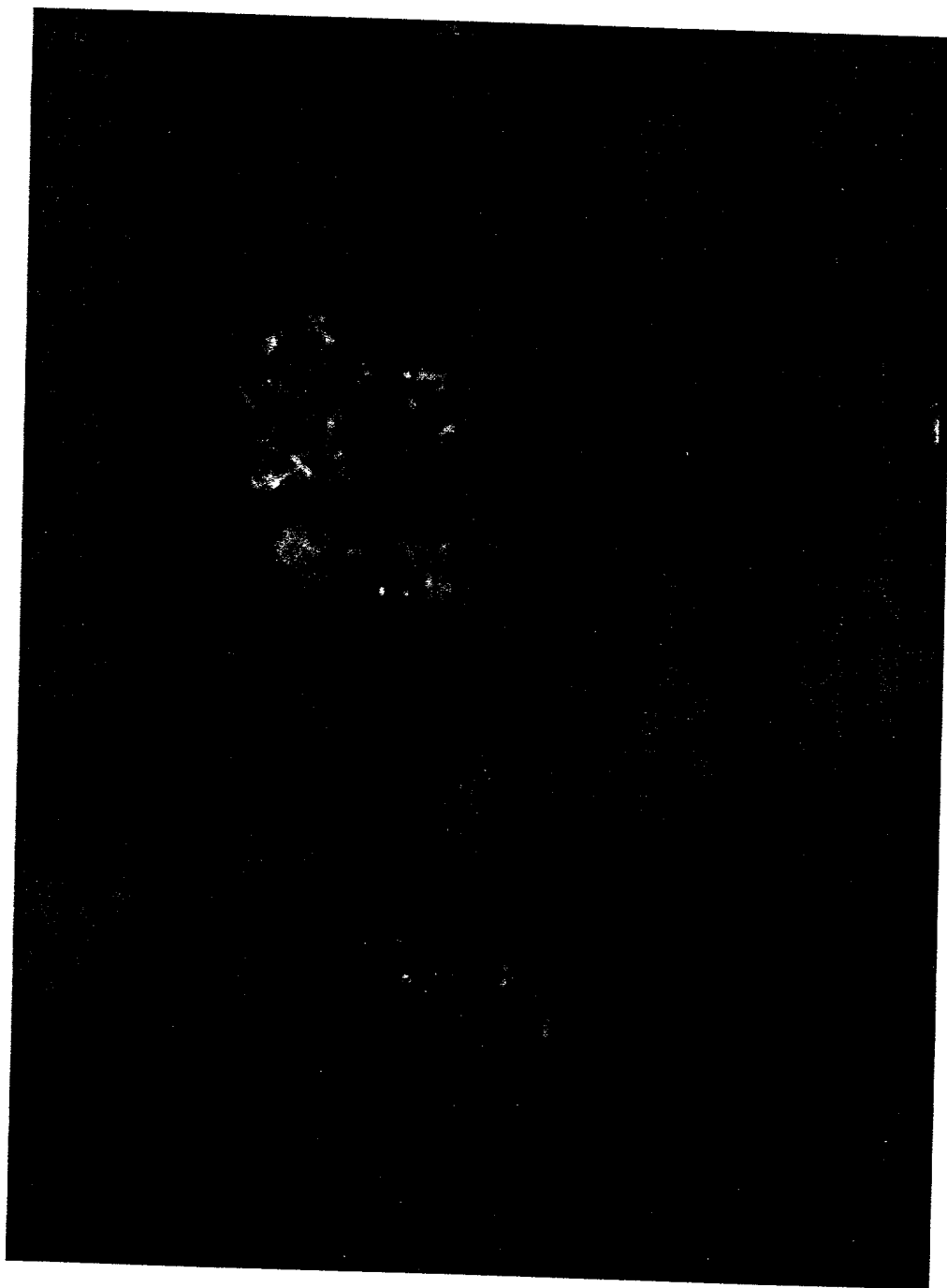
MODEL2

MESENCHYMAL NICHE

ENDOCRINE CELL

MORPHOGENS

STEM CELL

LUMEN

EPITHELIAL CELLS

DUCT

FIG. 12

FIG. 13A

FIG. 13B

FIG. 14

DAY: E8.5   E9.5   E13   E14   E15

                1st TRANSITION   2nd TRANSITION

                PCX-1   PCX-1   PDX-1
                GLUCAGON   Ngn-3   BETA2/neuroD
                (INSULIN)   NO HORMONES   Isl1
                                                           Pax6
                                                           Nkx6.1

                                                           β-cell

STEM   PROGENITOR   PROLIFERATION   DIFFERENTIATION
CELL   CELL

                                                           α-cell

                                                           BRAIN-4
                                                           BETA2/neuroD
                                                           Isl1
                                                           Pax6

PROGLUCAGON

NIPs+SCs   ISLETS

RT-PCR

SOUTHERN BLOT

FIG. 15A

INSULIN

NIPs+SCs   ISLETS

RT-PCR

SOUTHERN BLOT

FIG. 15B

FIG. 15C

NEURO-
ENDOCRINE

EXOCRINE

HEPATIC

SYN

AMY

TTR

HGFR

CARB

HGF

GLUT-2

E-CAD

XBP

AFP

FIG. 16

Figure 17

SEQ ID NO: 3

atggccggcgcccccggcccgctgcgccttgcgctgctgctgctcgggatggtgggcagggccggcccccgcccccagggtgccactg
tgtccctctgggagacggtgcagaaatggcgagaataccgacgccagtgccagcgctccctgactgaggatccacctcctgccacagact
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tgggccagcagtgtgccgcagggccacgtgtaccggttctgcacagctgaaggcctctggctgcagaaggacaactccagcctgccctg
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gtggctcttctaacctcagccaaacacagagcgggagtgacgggagcctcctctgcttgcatcacttggggtcaccaccctcccctgtcttct
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acttgggagcttgtatatacttcactcacttttctttattgctgtgaatagtctgtgtgcacaatgggcaattctgacttctcccatctagtggaaatg
agcgaaatcatggttgtagtgatgttg

SEQ ID NO: 4

MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPA
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NSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFRHLHCTR
NYIHLNLFASFILRALSVFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQ
YCVAANYYWLLVEGVYLYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYE
DEGCWTRNSNMNYWLIIRLPILFGIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLAKST
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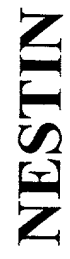KSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAGSSMYTATCQASCS

GLP-1R/NUC

NESTIN

GLP-1R

PRE-IMM

Figure 16A
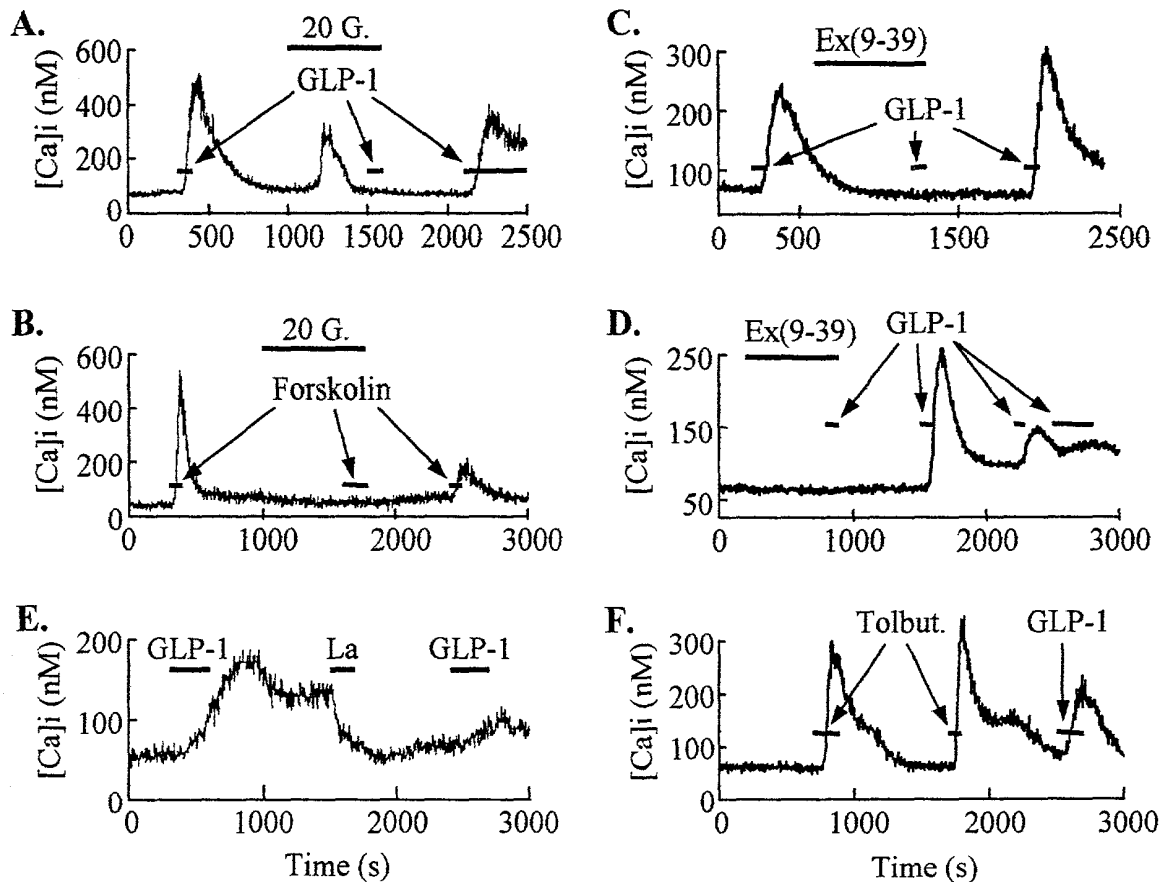
Figure 18

B

346bp

NIPs   Islets

Figure   GLP-1(7-36)amide and Tolbutamide stimulate [Ca^{2+}]$_i$ influx in stem cells.

(A) Fura 2 loaded cells bathed in 5.6 mM glucose show a [Ca^{2+}]$_i$ increase in response to 10 nM GLP-1. Increasing the extracellular glucose to 20 mM (20 G) also caused an increase of [Ca^{2+}]$_i$ but application of GLP-1 in 20 mM glucose failed to produce a [Ca^{2+}]$_i$ response. A third application of GLP-1 on returning to 5.6 mM glucose produced a [Ca^{2+}]$_i$ response. **(B)** The glucose-dependent effects of GLP-1 were reproduced by 10 mM forskolin, suggesting that [Ca^{2+}]$_i$ elevation is cAMP-mediated. **(C)** The GLP-1 mediated increase of [Ca^{2+}]$_i$ was reversibly inhibited by 10 nM exendin (9-39). This effect is not due to receptor desensitization **(D)** as application of GLP-1 in the presence of exendin (9-39) failed to produce a response whereas subsequent applications of GLP-1 after washout of exendin produced repeated [Ca^{2+}]$_i$ elevations. **(E)** The GLP-1-mediated increase of [Ca^{2+}]$_i$ is inhibited by 0.5 mM extracellular La^{3+}, suggesting that GLP-1 stimulates Ca^{2+} influx. **(F)** Stem cells bathed in 5.6 mM glucose were stimulated with 100 μM tolbutamide (Tolbut.) and respond to repeated applications with increases in [Ca^{2+}]$_i$. Application of 10 nM GLP-1 also stimulates an increase of [Ca^{2+}]$_i$ suggesting that GLP-1 acts by depolarizing the cells.
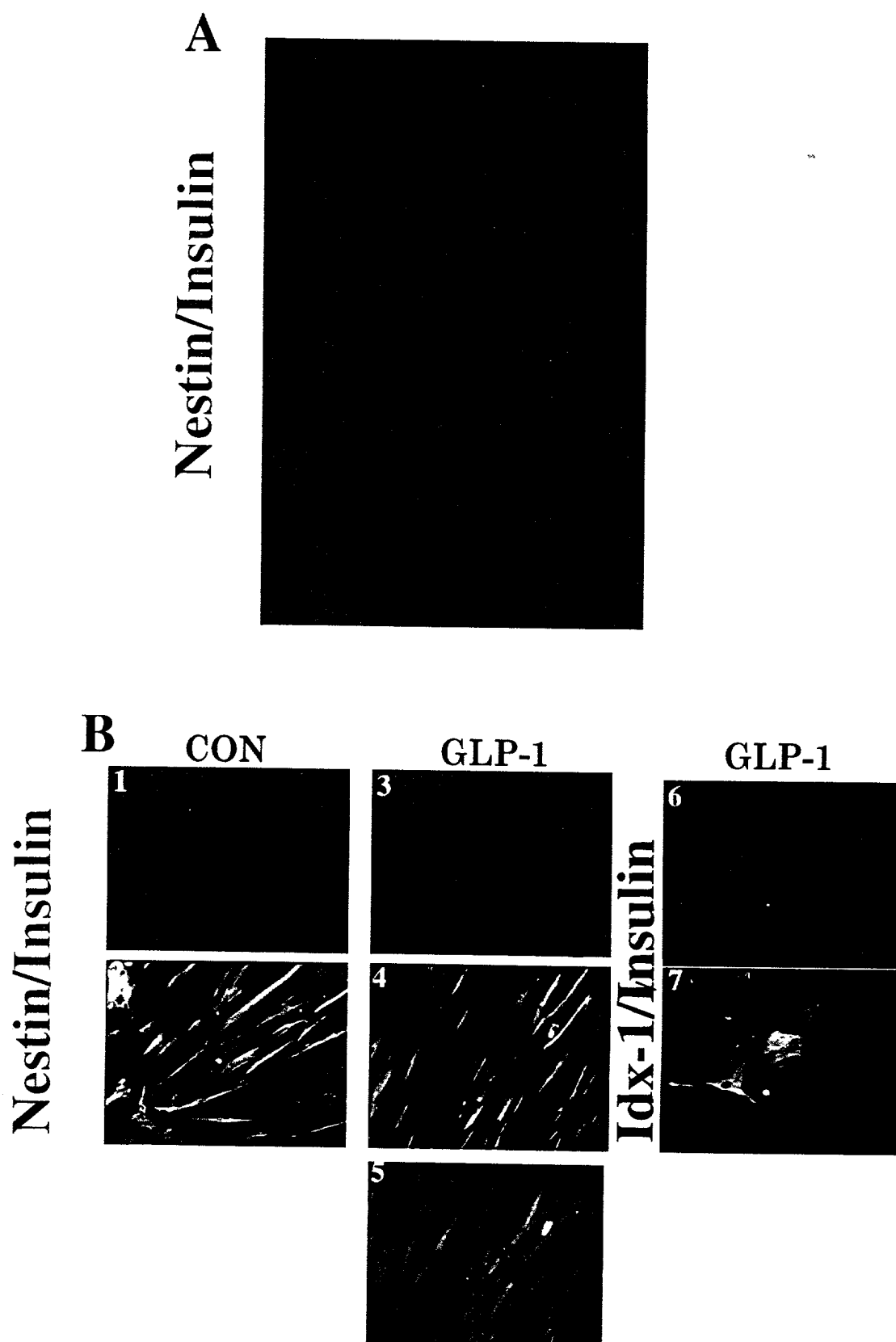
Figure 20

A

Nestin/Insulin

B

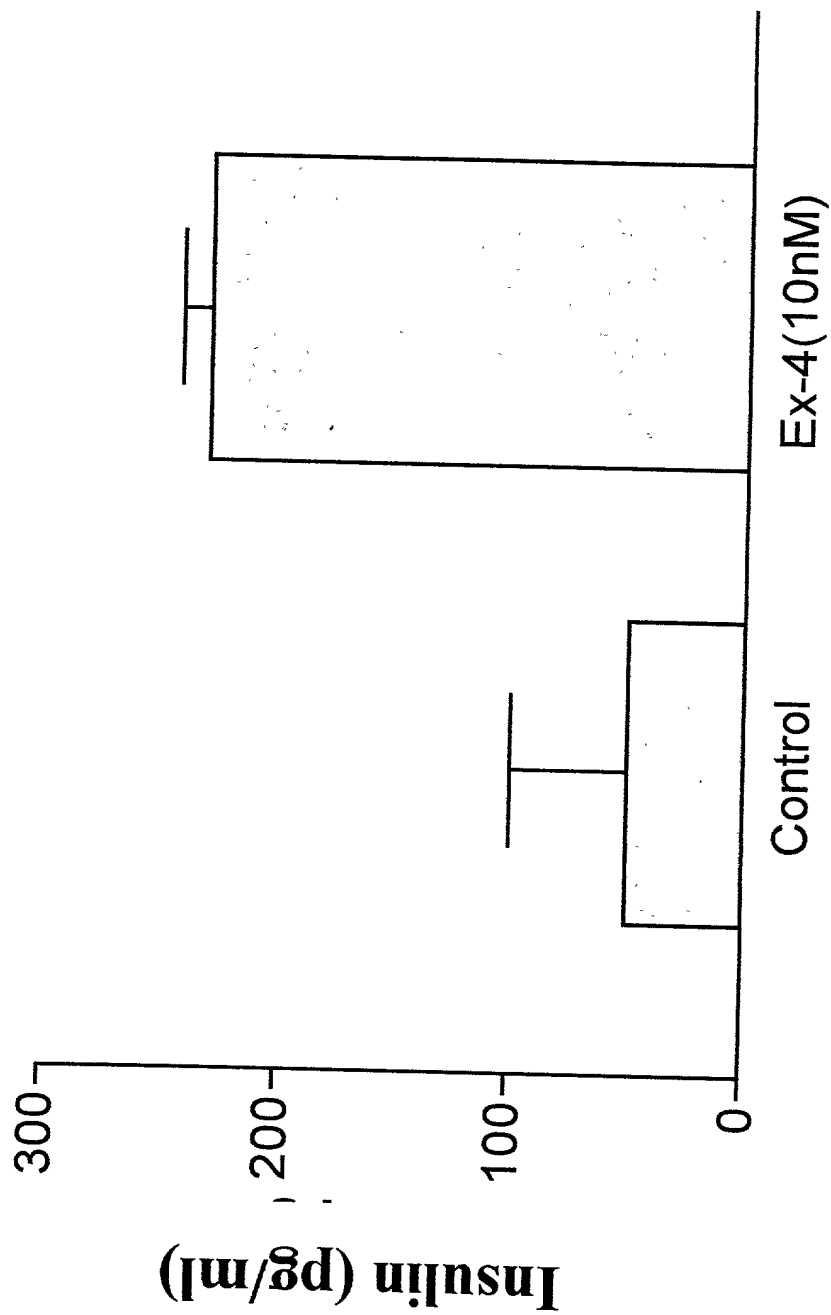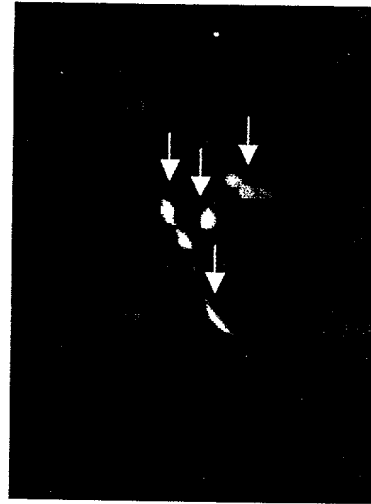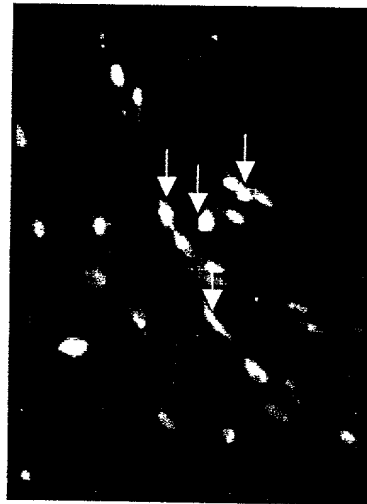CON   GLP-1   GLP-1

Nestin/Insulin   Idx-1/Insulin

Figure 21

Figure 22

A

Transfected with hIDX-1 and
incubated with GLP-1 (7-36)

B

Transfected with hIDX-1 and
incubated with Vehicle (PBS)

Insulin/ IDX